DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology

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Abstract: Dendritic cells (DC) are present in essentially every tissue where they operate at the interface of innate and acquired immunity by recognizing pathogens and presenting pathogen-derived peptides to T cells. It is becoming clear that not all C-type lectins on DC serve as antigen receptors recognizing pathogens through carbohydrate structures. The C-type lectin DC-SIGN is unique in that it regulates adhesion processes, such as DC trafficking and T-cell synapse formation, as well as antigen capture. Moreover, even though several C-type lectins have been shown to bind HIV-1, DC-SIGN does not only capture HIV-1 but also protects it in early endosomes allowing HIV-1 transport by DC to lymphoid tissues, where it enhances *trans* infection of T cells. Here we discuss the carbohydrate/protein recognition profile and other features of DC-SIGN that contribute to the potency of DC to control immunity. *J. Leukoc. Biol.* 71: 921–931; 2002.

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DENDRITIC CELLS

Dendritic cells (DC) are professional antigen presenting cells (APC) that are positioned throughout peripheral tissues to act as sentinels against invading pathogens [1]. Their journey starts in the bone marrow where stem cells differentiate and migrate as precursor DC into the blood (**Fig. 1**). From there, immature DC seed the tissues where they monitor for invading pathogens, which they capture and process to antigenic fragments. Upon pathogen capture, immature DC receive activation signals, which initiate their maturation and migration to secondary lymphoid organs in order to present the processed antigens to naïve T cells and induce antigen-specific immune responses. Both maturation and migration of DC are carefully orchestrated by panoplies of chemokines and adhesion molecules; chemokines control the differentiation stages of the DC and direct the migration of the various DC subtypes [2]. Once in the T cell area of lymph nodes, chemokines attract naïve T cells toward the DC, enabling maximal exposure of the major histocompatability complex (MHC)-presented peptide repertoire to the T cells (Fig. 1). Adhesion molecules are crucial for all cellular interactions of the DC during its journey from bone marrow into blood, from blood into the peripheral tissues, and subsequently into lymphoid tissues. Adhesion receptors and costimulatory molecules enable the establishment of contact between naïve T cells and DC, providing sufficient stability to allow scanning of the MHC class II-peptide complexes and to induce T cell receptor (TCR) triggering. Recently, many new cell-surface molecules have been identified on DC that may contribute to their function in controlling innate as well as adaptive immunity. In particular, a large diversity of C-type lectins has been identified on DC: some of these regulate pathogen recognition while others regulate signaling or cellular interactions such as DC migration and T-cell binding. This illustrates that C-type lectins regulate many DC functions involved in establishing innate and adaptive immunity. Some of these C-type lectins are not expressed exclusively by DC, but are also expressed by macrophages that participate in immune activation, whereas others are DC-specific and mediate only DC functions.

DC-SIGN, A DC-SPECIFIC C-TYPE LECTIN

Two years ago, we identified DC-SIGN, a novel DC-specific adhesion receptor on human DC, which is essential in several key functions throughout the life cycle of DC [3]. DC-SIGN was discovered by the observation that DC bind the intercellular adhesion molecule (ICAM)-3 (CD50) with very high affinity. Although ICAM-3, a member of the immunoglobulin (Ig) superfamily, was known to be a ligand for the β2 integrins lymphocyte function-associated antigen-1 (LFA-1; αLβ2) and αDβ2 [4], these receptors did not contribute to the binding activity of ICAM-3 by DC. By immunizing mice with human DC, we generated antibodies that blocked DC binding to ICAM-3 and thus identified a novel adhesion receptor on DC, which was named DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN). These antibodies were used to further functionally characterize DC-SIGN [3].

DC-SIGN (CD209) is a type II transmembrane protein that, based on its structure, belongs to the C-type lectin family [3, 5]. DC-SIGN contains a short, cytoplasmic N-terminal domain with several intracellular sorting motifs, an extracellular stalk of seven complete and one partial tandem repeat, and a C-

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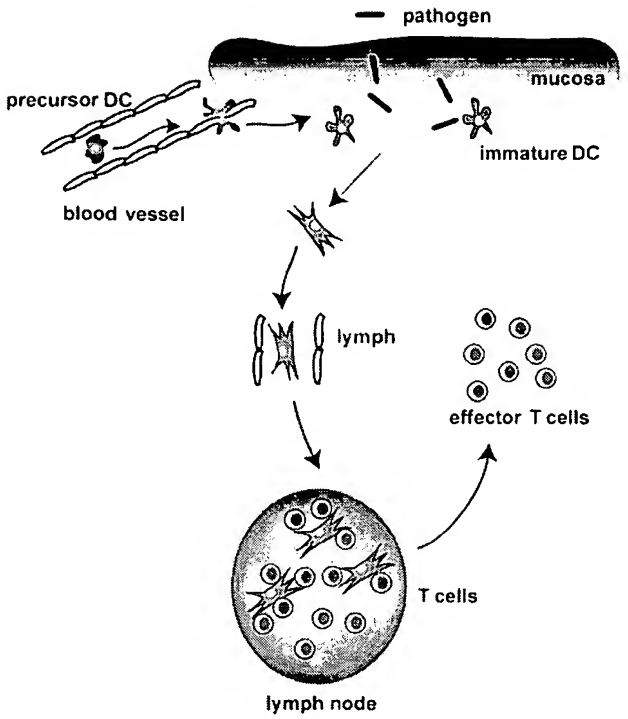

Fig. 1. Role of DC in the immune system. Precursor DC migrate from blood into tissues to monitor these tissues against invading pathogens. Upon entry, pathogens are sampled by these immature DC, resulting in maturation and migration of the maturing DC through the lymph into the lymphoid tissues. Pathogen-derived antigens are presented by the mature DC to naïve T cells, and antigen-specific T cells are activated following TCR triggering. These T cells proliferate and migrate into the compromised tissues to perform their effector functions.

terminal lectin or carbohydrate-recognition domain (CRD) [3, 5]. Many C-type lectins have been identified on DC: Type I multi-CRD lectins are represented by the mannose receptor (MR) [6] and DEC-205 [7], and type II single-CRD lectins, by DC-SIGN, dectin-1, dectin-2, Langerin, BCDA-2, DCIR, DLEC, CLEC-1, and DC-ASGPR [3]. Within the CRD, the highly conserved EPN or QPD sequences are essential in recognizing mannose- and galactose-containing structures, respectively. Most C-type lectins expressed by DC have specificity for mannose-containing carbohydrates; however, each C-type lectin may recognize a unique branching and positioning of mannose residues on a given pathogen or cell-surface structure. For example, the MR recognizes branch-end mannose residues, whereas DC-SIGN recognizes high-mannose residues located more internally within a glycan structure [8]. Most C-type lectins on DC are pathogen recognition receptors that capture and deliver pathogens or their fragments into the antigen processing compartments that permit MHC class II-restricted presentation. Although the natural ligands and the carbohydrate-specific recognition of most C-type lectins have not yet been identified, it is hypothesized that these C-type lectins regulate the capture of many different pathogens, increasing the diversity and efficiency of antigen recognition by DC.

Many C-type lectins are not expressed exclusively on DC but are also expressed by other APC such as macrophages. DC-SIGN is expressed by DC in vitro and in vivo [4]. It is expressed abundantly on monocyte- and $CD34^+$-derived DC, as well as in vivo dermal DC of the skin, but not by Langerhans cells in the epidermis [3]. In vivo, DC-SIGN is expressed by immature DC in peripheral tissue as well as on DC present in lymphoid tissues such as lymph nodes, tonsils, and spleen [3]. Two DC-SIGN-positive DC precursor populations that differ in CD14 expression were found to be present in peripheral blood [9]. Moreover, DC-SIGN expression has also been demonstrated on specialized APC such as decidual macrophages and Hofbauer cells in the placenta [10, 11] and on alveolar macrophages in the lung [12, 13].

Our initial finding that DC-SIGN is a DC-specific adhesion receptor that mediates DC binding to ICAM-3 initiated our search for other ligands, in particular those that share structural identity with ICAM-3—two other members of the Ig superfamily, ICAM-1 (CD54) and ICAM-2 (CD102). Strikingly, DC-SIGN binds ICAM-2 but not ICAM-1, demonstrating a highly regulated recognition of its Ig ligands [9]. The inhibition of DC-SIGN function by mannan suggests that its interaction with its ligands is mediated by mannose-like carbohydrates [3]. Indeed, recent studies demonstrated that DC-SIGN can interact with high mannose-type oligosaccharides but not with single terminal mannose residues, which are recognized by the MR [3, 14]. The natural ligand of DC-SIGN on lymphocytes, ICAM-3, contains N-linked glycosylations consisting of high mannose-type oligosaccharides [15], and, indeed, enzymatic removal of the N-linked carbohydrates from ICAM-3 abrogates its binding to DC-SIGN completely [16]. Although DC-SIGN has been demonstrated to interact specifically with unique purified oligosaccharide structures [14], the specific carbohydrate structure it recognizes on ICAM-3 has yet to be identified. Future research will determine what particular glycosylation site is recognized on ICAM-3 and how the glycosylation is regulated to provide recognition by DC-SIGN.

DC-SIGN MEDIATES MIGRATION OF DC FROM BLOOD INTO TISSUES

A fundamental aspect of DC function in controlling immunity is its capacity to migrate, providing a continuous surveillance for incoming foreign antigens and a prompt response to present the encountered antigens to T cells [1]. Precursor and immature DC migrate from the blood into peripheral tissues in order to replenish resident DC or in response to inflammatory signals. The egress from blood into tissues is regulated tightly and is mediated by a multistep process that involves leukocyte rolling, rapid activation of leukocyte, adhesion to endothelial ligands through activated integrins, and diapedesis [17]. The interaction of leukocytes with the endothelial cells lining the blood vessels is an important control point in the egress. Rolling along the endothelial linings is mediated by the selectins on endothelial and leukocytes; selectins are also C-type lectins, which bind to specific carbohydrates such as sialyl Lewis X (sLex) expressed on specialized cell-surface receptors (**Fig. 2**). It is striking that we demonstrated that DC-SIGN mediates the tethering and rolling of DC-SIGN-positive cells along ICAM-2-expressing surfaces [9]. ICAM-2 is expressed

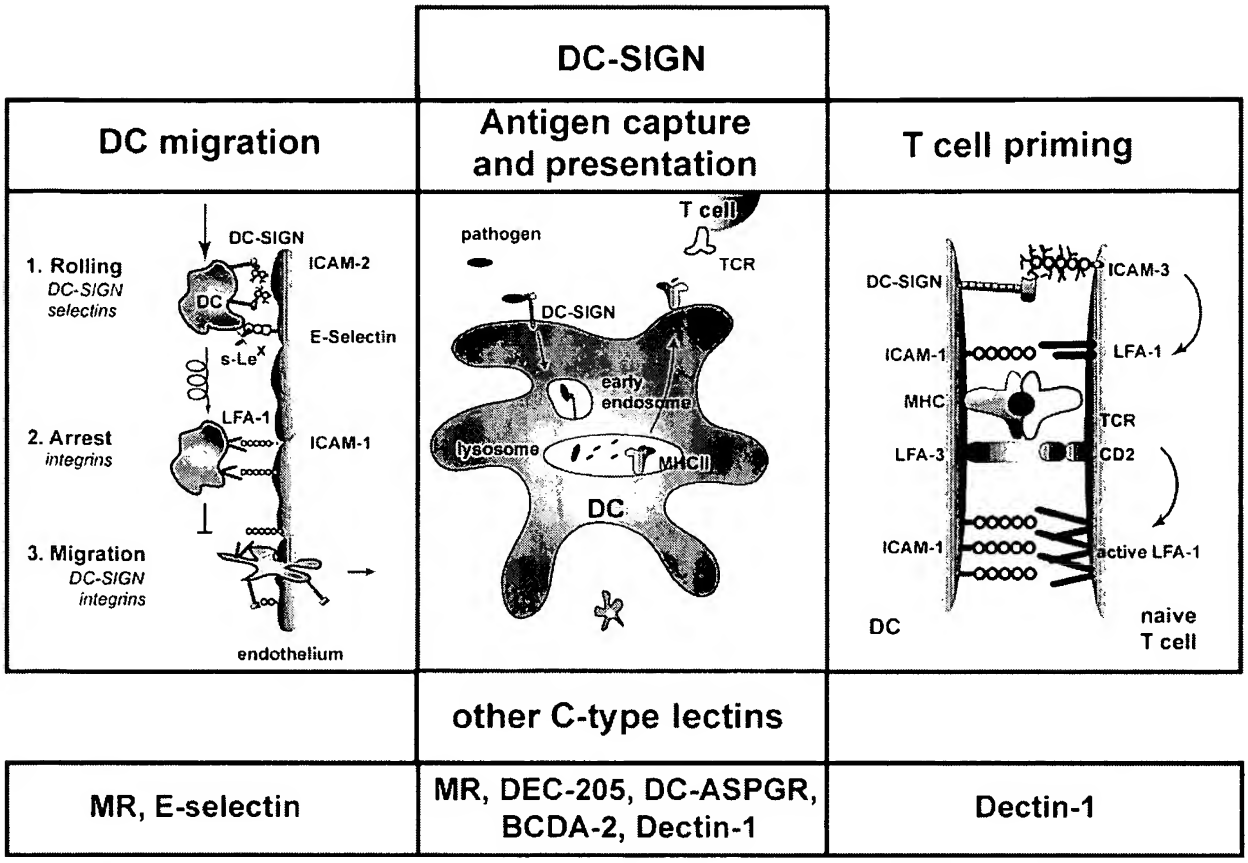

Fig. 2. DC-SIGN controls many functions of DC to elicit immune responses. The egress of precursor DC from blood into tissues is mediated partly by DC-SIGN. DC-SIGN facilitates rolling and transendothelial migration of DC-SIGN$^+$ precursor DC, whereas arrest is mediated by integrin-mediated interactions. DC-SIGN also functions as an antigen receptor. DC-SIGN internalizes rapidly upon binding soluble ligand and is targeted to late endosomes/lysosomes, where antigens are processed and presented by MHC class II molecules. Moreover, initial DC-T-cell clustering, necessary for an efficent immune response, is mediated by transient interactions between DC-SIGN and ICAM-3. This interaction facilitates the formation of low-avidity LFA-1/ICAM-1 interaction and scanning of the antigen-MHC repertoire. It is becoming clear that other C-type lectins also participate in these processes. Selectins and the MR may regulate DC migration. Many other C-type lectins on DC, such as MR, DEC205, DC-ASPGR, BCDA-2, and dectin-1, are antigen receptors that recognize various distinct carbohydrate-containing antigens. It has been postulated that dectin-1 regulates T-cell priming, however its interaction with T cells is not carbohydrate-dependent.

constitutively on the endothelium of blood and lymphatic vessels as well as on high endothelial vascular cells and leukocytes. ICAM-2 plays a central role in mediating leukocyte recirculation as well as homing into secondary lymphoid tissues. Although DC-SIGN binds to ICAM-2 and ICAM-3 under static conditions, only the DC-SIGN-ICAM-2 interaction resists shear stresses encountered under physiological flow conditions [9]. It is interesting that DC-SIGN behaves as a DC-specific rolling receptor for ICAM-2 and is functionally similar to the selectins, which are well known for their regulation of leukocyte rolling on carbohydrate-structure recognition (Fig. 2) [18].

Besides having a function in the rolling of DC along blood vessel linings, DC-SIGN also mediates the adhesion of DC to endothelium via ICAM-2 and their subsequent transendothelial migration [9], steps 2 and 3 in the multistep paradigm, respectively (Fig. 2). Blocking antibodies against DC-SIGN inhibit the migration of DC across resting endothelium, whereas a combination of blocking antibodies against DC-SIGN and β1 and β2 integrins abrogates migration across activated endothelium completely [9]. DC also expresses LFA-1, and this β2 integrin has a high affinity for ICAM-1 and a lower affinity for ICAM-2 [4]. Resting endothelial cells express low levels of ICAM-1, but after activation, ICAM-1 is up-regulated and bound by LFA-1. This explains the contribution of β2 integrins in the interaction of DC to activated endothelium [9]. Moreover, vascular cell adhesion molecule-1 is up-regulated, indicating that very late antigen-4 (VLA-4) also participates [9]. Thus, DC-SIGN plays a role in the migration of DC-SIGN-positive DC from blood into tissues in order to replenish resident DC or in response to inflammatory signals (Fig. 2).

DC-SIGN is up-regulated rapidly on monocytes in the presence of granulocyte macrophage-colony stimulating factor and interleukin-4 [3]; thus, DC-SIGN up-regulation by cytokine mediators in response to inflammation may induce migration of precursor DC from blood into the periphery. The presence of DC-SIGN-positive DC precursors in blood further supports the hypothesis that, under physiological circumstances, DC-SIGN-ICAM-2 interactions mediate rolling along endothelial linings, a function that is potentially important prior to transmigration of DC into the periphery [9]. These DC-SIGN-positive DC precursors could be poised to exit the blood at inflammatory sites, allowing rapid recruitment of these cells to sites where their surveillance function is needed. The high expression of DC-SIGN on immature DC in the peripheral tissues and the expression of ICAM-2 on lymphatic vessels further support a central role for the DC-SIGN-ICAM-2 interaction in DC-specific migration from blood into the periphery, whether inflamed or not, and subsequently, via the lymph into lymphatic tissues [4, 9]. Recently, it has been reported that the MR, another C-type lectin, can recognize sLex structures presented on the endothelial cells and could thus contribute to the rolling interactions of DC [19]. The MR has been shown also to be expressed on lymphatic endothelial cells where it can interact with L-selectin to mediate lymphocyte binding [20]. This illustrates that several lectin receptors, such as DC-SIGN, selectins, and possibly the MR, regulate rolling and migration, processes dependent on recognition of appropriate carbohydrate structures. While DC-SIGN probably recognizes a high-mannose structure on ICAM-2 present on endothelial cells, the MR and selectins mediate rolling upon recognition of endothelial receptors that present sLex structures. Tissue-restricted glycosylation of ICAM-2 or presentation of sLex may determine and regulate migration of DC to particular sites. Recently, tissue-restricted glycosylation has been demonstrated to regulate selectin-mediated, site-specific migration of leukocytes [21].

DC-SIGN FUNCTIONS AS AN ANTIGEN RECEPTOR

In addition to DC-SIGN, DC express several other C-type lectins, such as the MR [6], DEC-205 [7], BCDA-2 [22], and DC-ASGPR [23], which function as pathogen recognition receptors. These lectins interact with conserved molecular patterns shared by a large group of microbes and internalize these pathogens for processing and antigen presentation, thus initiating immune responses against a diversity of micro-organisms [24]. For example, the dectin-1/β-glucan receptor has a high affinity for β-glucans and binds pathogens such as *Saccharomyces cerevisiae* and *Candida albicans* [8], whereas the MR has multiple CRD that display a high affinity for terminal mannose residues on a variety of pathogens and antigens. This indicates a possible role for this receptor in the innate immunity [25, 26]. The MR internalizes ligands constitutively into early endosomes, where bound ligand is released, and recycles back to the cell surface, thus resulting in high amounts of internalized antigens and efficient antigen presentation by DC [27, 28]. This process is mediated by a tyrosine-based motif in the cytoplasmic tail of the mannose receptor. In contrast, DEC-205 seems to internalize antigens to deeper endosomal compartments [7]. That DEC-205 internalizes antigens to more acidic compartments is a result of different amino acid motifs present in its cytoplasmic tail. In particular, a triacidic cluster allows it to internalize to lysosomes. In contrast to the MR, it is not yet known which carbohydrate and pathogen structures are recognized and internalized by DEC-205. The cytoplasmic tail of DC-SIGN contains three putative internalization motifs, and we have recently shown that DC-SIGN can function as an endocytic receptor (Fig. 2) [29]. Binding of soluble ligand to DC-SIGN induces rapid internalization from the cell surface mediated by a dileucine motif. Similar to DEC-205, DC-SIGN contains a triacidic cluster in its cytoplasmic tail, and accordingly, DC-SIGN-ligand complexes are targeted to lysosomal compartments where ligands are processed for MHC class II presentation to T cells, indicating an important function for DC-SIGN as an antigen receptor (Fig. 2) [29]. In contrast, BCDA-2 and dectin-2 do not contain any cytoplasmic internalization motifs, yet, upon cross-linking, these receptor are internalized; their cytoplasmic tails may associate with adaptor proteins, which themselves have signaling capacity [30]. This indicates that the many C-type lectins expressed by DC can capture and process antigen. However, it is likely that each of these C-type lectins has distinct recognition profiles to bind specific ligands and pathogens, and certain pathogens might interact with more than one C-type lectin. The variety of C-type lectin receptors on DC may be essential to elicit immune responses against a broad spectrum of pathogens, and it will be interesting to determine whether they can differently affect the outcome of an immune response.

DC-SIGN IS INVOLVED IN THE INITIATION OF AN IMMUNE RESPONSE

An immune response is initiated when the TCR on T cells recognizes peptides bound to MHC class II molecules on the surface of APC such as DC. The initial interaction of T cells with DC is antigen-independent and allows scanning of the peptide-MHC class II complex repertoire by the TCR. This requires the formation of a specialized junction between these cells, the immunological synapse, which is generated by the carefully orchestrated recruitment of specific adhesion receptors into the contact site to strengthen DC-T cell contact (Fig. 2) [31–33]. The abundance of appropriate MHC-peptide complexes is too low to mediate significant adhesion by itself, and thus adhesion molecules are essential for an efficient TCR engagement. The initial interaction is transient and allows rapid scanning of the MHC-peptide complexes by T cells. Several studies have suggested that ICAM-3, which is expressed at high levels on resting T cells, might be important in establishing these initial DC-T cell interactions [3, 34–38]. Recently, Montoya et al. [38] demonstrated that ICAM-3 is recruited in the contact region of APC with T cells. The observed clustering occurred rapidly after cell-cell contact, supporting a role for ICAM-3 in the early adhesive events. The counter-receptor for ICAM-3 on DC was originally thought to be LFA-1. However, the β2 integrin LFA-1 is inactive on DC

and is able to interact with ICAM-3 with a low affinity only after activation [3]. The discovery of DC-SIGN increased our understanding of the initiation and regulation of DC-T cell interactions. DC-SIGN has a high affinity for ICAM-3 and is the primary receptor for this molecule on DC, as antibodies against DC-SIGN inhibit the DC-ICAM-3 interaction completely [3]. Moreover, DC-SIGN is fully active on DC and does not need to be activated in contrast to LFA-1. The importance of DC-SIGN-ICAM-3 interactions in the initial DC-T-cell contact is further emphasized by the potency of anti-DC-SIGN antibodies to inhibit DC-T cell clustering and DC-induced proliferation of resting T cells. The interaction of DC-SIGN with ICAM-3 is transient, allowing screening of the MHC-peptide complexes [3]. We propose a model in which the initial interaction of DC with resting T cells is mediated by the DC-SIGN-ICAM-3 interaction, followed by more stable DC-T cell contacts mediated through other adhesion molecules such as LFA-1 and LFA-3 (Fig. 2). The initial DC-SIGN-ICAM-3 contacts stabilize intimate DC-T cell membrane contact transiently to enable efficient TCR engagement. The transient nature of the DC-SIGN-ICAM-3 interactions enables DC to interact with a large number of resting T cells until productive TCR engagement is obtained. TCR signaling increases the avidity of LFA-1 and CD2, thereby strengthening the interaction between DC and T cell via multiple adhesive contacts through LFA-1 and LFA-3 that provide further positional stability and full activation of the T cell by the DC (Fig. 2) [4, 33]. To date, DC-SIGN is the only C-type lectin on DC that exhibits a strong adhesion function. Recently, murine dectin-1 and its human homologue the β-glucan receptor [39, 40] have been mentioned as C-type lectins that regulate T cell binding and/or costimulation of T cells. However, the mechanism by which the β-glucan receptor interacts with T cells is unknown.

DC-SIGN AS A NOVEL HIV-1 RECEPTOR

The identification of DC-SIGN as a DC-specific adhesion receptor [41] revealed its 100% identity to the previously cloned HIV-1 envelope-binding C-type lectin [5] and initiated a detailed investigation into the function of DC-SIGN as an HIV-1 receptor on DC [41]. Early work revealed that DC pulsed with HIV-1 promote a robust infection of cocultivated T cells [42, 43]. This property of DC to enhance infection of T cells was defined molecularly 8 years later when DC-SIGN was identified as the HIV-1 *trans* receptor on DC [41]. The affinity of DC-SIGN for the HIV-1 envelope glycoprotein gp120 exceeds that of CD4 [5], but, in contrast to CD4, DC-SIGN does not function as a classical HIV-1 entry receptor; coexpression of DC-SIGN with CD4 or CCR5 does not enable HIV-1 entry into HIV-1 nonpermissive cells [41]. In contrast, DC-SIGN acts as an HIV-1 *trans* receptor that binds HIV-1 and transmits it very efficiently to neighboring permissive target cells [41]. DC-SIGN expressed on DC and on transfectants binds RS- and X4-tropic HIV-1, HIV-2, and simian immunodeficiency virus and transmits these viruses to recipient T cells resulting in an efficient infection of T cells [41]. So far, no differences in affinity of DC-SIGN for R5- or X4-tropic strains have been demonstrated, which could explain the selective infection by R5-tropic strains in sexual transmission of HIV-1 [44]. Strikingly, DC-SIGN does not only capture and transmit HIV-1, but also enhances infection of T cells; at low virus titers, CD4/CCR5-expressing cells are not detectably infected without the assistance of DC-SIGN in trans [41]. Conditions in which the number of HIV-1 particles is limiting are likely to resemble those found early during infection in vivo, which suggests that DC-SIGN is required not only for HIV-1 transmission from mucosa to lymphoid tissues, but also for efficient infection of T cells. The binding of HIV-1 to DC-SIGN alone is not sufficient for transmission of HIV-1, as HIV-1 binding and transfer by DC-SIGN have been shown to be dissociated functions [45].

To date, DC-SIGN is unique in this function as it not only binds HIV-1 gp120, but also enhances the HIV-1 infection of T cells in trans [41]. The process through which DC-SIGN promotes efficient infection in trans of cells through their CD4/chemokine receptor complex remains unclear. Binding of the viral envelope glycoprotein to DC-SIGN may induce a conformational change in gp120 that enables a more efficient interaction with CD4 and/or the chemokine receptor and subsequent membrane fusion with T cells. Alternatively, binding of viral particles to DC-SIGN may focus or concentrate the virus particles at the surface of the DC and may thus increase the probability that entry will occur after binding to the CD4 and coreceptor complex on target cells. The potency of DC-SIGN to capture and transmit HIV to T cells may largely depend on the membrane organization of DC-SIGN in rafts or its capacity to multimerize [17]. Future experiments will determine the molecular mechanism by which DC-SIGN enhances the infection of T cells and will elucidate whether a diversity in multimerization and membrane organization of DC-SIGN is instrumental for its function as an HIV-1 *trans* receptor.

Whereas DC-SIGN-bound ligand is internalized for processing in degradation compartments, we previously demonstrated that HIV-1 bound to DC-SIGN is remarkably stable and retains infectivity for prolonged periods (**Fig. 3**) [41]. Our initial experiments, attempting enzymatic digestion of cell surface-bound HIV-1, demonstrated that HIV-1 is protected and probably "hides" within the cell close to the cell membrane without being degraded. In agreement with these results, Kwon et al. [46] shows in a recent paper that HIV-1 is indeed internalized upon binding to DC-SIGN into nonlysosomal acidic organelles (Fig. 3). Neutralizing the pH of these compartments or preventing internalization by deletion of the cytoplasmic tail of DC-SIGN abrogates DC-SIGN-mediated, enhanced *trans* infection of T cells, indicating that internalization of HIV-1 is crucial for DC-SIGN-mediated enhancement of the infection of T cells [46]. The question still remains as to how intact HIV-1 virions escape targeting to lysosomes as occurs for other DC-SIGN-ligands [29] and how they can protect themselves against processing and presentation. It is interesting that in mature DC, DC-SIGN is targeted to early endosomal compartments in which HIV-1 would be protected against degradation [29], suggesting that maturation of DC by HIV-1 may lead to its altered internalization. Identification of the mechanism by which HIV-1 prevents degradation and remains highly infectious may lead to the development of successful strategies to combat HIV-1 dissemination. Moreover, finding a way to over-

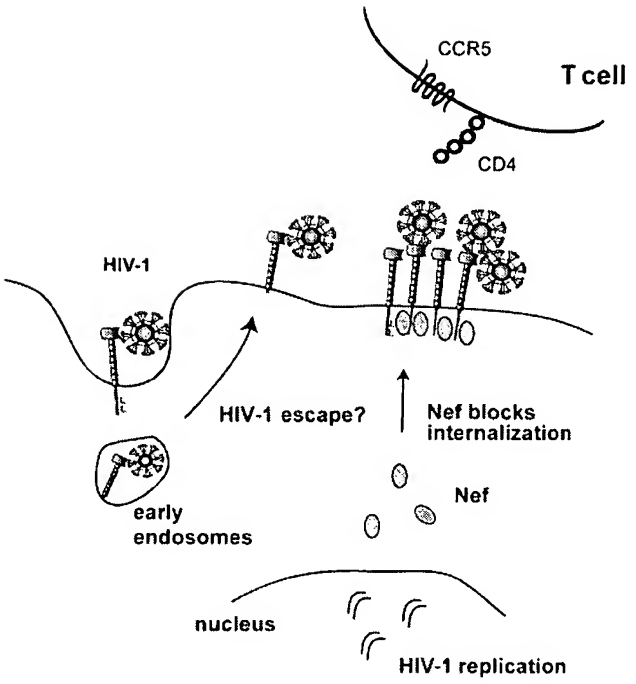

Fig. 3. HIV-1 interferes with the internalization pathway of DC-SIGN, resulting in enhanced *trans* infection of T cells. DC-SIGN binding stabilizes infectious HIV-1 and enhances *trans* infection of T cells. DC-SIGN can function as an antigen receptor, but HIV-1 escapes deep internalization and processing by preventing targeting to late endosomes. Moreover, HIV-1 Nef blocks internalization of DC-SIGN, possibly by interacting with the dileucine motif in the cytoplasmic tail of DC-SIGN, resulting in an increased cell-surface expression and increased HIV-1 transmission to T cells.

ride this mechanism to target internalized DC-SIGN-HIV complexes to lysosomes would greatly facilitate HIV-1 processing in DC and would enhance specific anti-HIV-1 immune responses while reducing infection of T cells [29, 46].

To transmit HIV-1 to T lymphocytes, virions internalized by DC-SIGN should recycle back to the cell surface in order to contact entry receptors on the target cell [41]. The mechanism of virus recycling and whether this process is constitutively active or regulated remain to be determined. Interestingly, we have recently shown that the Nef protein of HIV-1 affects the intracellular trafficking of DC-SIGN (Fig. 3). Nef is crucial for viral replication, including DC-T cell binding [47]. In addition, Nef can interact with the cell-sorting machinery to down-regulate expression levels of CD4 and MHC class I and thus facilitate immune evasion [48]. Expression of Nef in immature DC results in a redistribution of DC-SIGN to the cell surface, thus reducing DC-SIGN internalization in favor of cell surface expression and facilitating increased cell adhesion and virus transmission to T cells [49]. Redistribution of DC-SIGN requires the dileucine motif in the cytoplasmic tail of DC-SIGN as well as a dileucine motif in Nef, indicating that Nef interferes with recognition of DC-SIGN by the sorting machinery (Fig. 3) [49]. The relocalization of DC-SIGN to the cell surface by Nef could play an important role in recycling DC-SIGN-internalized HIV particles to allow transmission to target T cells.

DC-SIGN INTERACTION WITH ICAM-3 IN CONTRAST TO gp120 IS CARBOHYDRATE-DEPENDENT

Detailed information about the mechanism of ligand binding is crucial for the development of specific DC-SIGN-based inhibitors and/or vaccines. The ICAM-3 binding activity of DC-SIGN is calcium-dependent, and DC-SIGN binds two Ca^{2+} ions [3, 5]: one calcium ion is essential for the tertiary structure, and the other calcium coordinates ligand binding [8, 16], similar to other C-type lectins [24]. The interaction of DC-SIGN with HIV-1, ICAM-2, and ICAM-3 is blocked by the polycarbohydrate mannan [3, 9, 41].

The HIV-1 envelope glycoprotein gp120 is also heavily glycosylated and contains high mannose-type oligosaccharides [50, 51]. Strikingly, although glycosylation seems to enhance the affinity of gp120 binding, neither O- nor N-linked glycosylations are vital for the interaction of the HIV-1 gp120 with DC-SIGN, as DC-SIGN interacts with enzymatically deglycosylated and nonglycosylated gp120 [16]. Therefore, the interaction of DC-SIGN with gp120 differs from that with ICAM-3, indicating different binding sites for ICAM-3 and HIV-1 gp120 in DC-SIGN; the C-type lectin DC-SIGN interacts with its ligands through carbohydrate interactions, such as those with mannan and ICAM-3, or through protein interactions, such as with gp120. However, the polycarbohydrate mannan is able to inhibit ICAM-3 and gp120 binding to DC-SIGN by occupying the binding site of DC-SIGN, suggesting that the carbohydrate and protein interactions to DC-SIGN are mediated by overlapping but distinct binding sites in DC-SIGN.

The interaction of ICAM-2 with DC-SIGN is also mediated by glycosylation similar to ICAM-3 and suggests a similar interaction in the different ICAM molecules (T.G., unpublished results). This is demonstrated further by the observation that DC-SIGN interacts with the second Ig domain of ICAM-2 and ICAM-3 (T.G., unpublished results). It is striking that DC-SIGN-positive cells are able to tether to and roll on ICAM-2 in contrast to ICAM-3 surfaces [9]. Thus, DC-SIGN interacts differently with ICAM-2 than with ICAM-3 even though the interactions share similar features. Possibly, the recognized carbohydrate structure and different size of the ICAM molecules determine the manner of interaction.

THE STRUCTURE OF DC-SIGN AND ITS DISTINCT BINDING SITES FOR gp120 AND ICAM

Recent elucidation of the three-dimensional structure of DC-SIGN cocrystallized with an oligosaccharide identified several important features of ligand binding by DC-SIGN [8]. Part of the pentasaccharide ligand forms coordination bonds with the Ca^{2+} at the principal site 2 [8]. Carbohydrate binding by DC-SIGN is mediated by the amino acid residues that are also in close contact with this Ca^{2+} and that form the core of the carbohydrate binding site (**Fig. 4**). This type of binding is a hallmark of C-type lectin-carbohydrate interactions [52]. How-

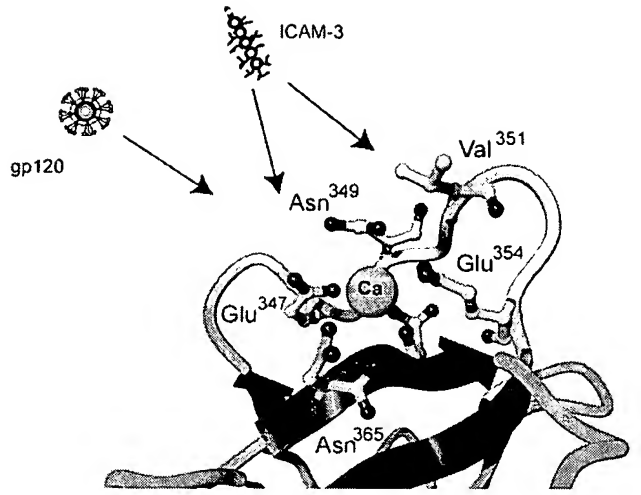

Fig. 4. The primary ligand-binding site in DC-SIGN is located at the top of the C-type lectin domain and includes the Ca^{2+} at site 2. Shown is the molecular model of the ligand-binding site, the Ca^{2+} site 2, and the Val^{351} amino acid residue in DC-SIGN. The four amino acid residues, Glu^{347}, Asn^{349}, Glu^{354}, and Asn^{365}, a coordinate binding of the Ca^{2+} ion, and all contribute to the binding of the ligand through hydrogen bonding via acidic or amide groups. The Val^{351} amino acid residue is involved in ICAM-3 but not in gp120 binding.

ever, the DC-SIGN interaction with carbohydrate ligands also has some unique characteristics compared with that of other C-type lectins [8]. Most C-type lectins, such as mannose binding protein A, interact with a single terminal mannose or GlcNAc residue in an oligosaccharide ligand, and the rest of the oligosaccharide points away from the surface of the protein [8]. In contrast, DC-SIGN binds an internal mannose of the oligosaccharide, and the external saccharides also interact with the surface of DC-SIGN [8]. Although the three-dimensional structure of DC-SIGN with an oligosaccharide revealed several important features of DC-SIGN binding [8], the interaction with its natural ligands is more complex as it is not only mediated by carbohydrate structures [16].

Even though the nature of the interaction of DC-SIGN with gp120 differs from that with ICAM-3, site-directed mutagenesis demonstrates that gp120 and ICAM-3 binding by DC-SIGN is mediated by the Ca^{2+} at site 2 and nearby amino acid residues that also serve as Ca^{2+} ligands (Fig. 4) [16]. The auxiliary Ca^{2+} bound at site 1 probably correctly orients the primary binding site, and indeed, removal of this Ca^{2+} by mutagenesis abrogates ICAM-3 and HIV-1 binding completely [16]. The importance of the calcium ions is underscored by the finding that the epitopes of the blocking antibodies against DC-SIGN and AZN-D1 and -D2 are located at the Ca^{2+} sites [16].

The differences in carbohydrate dependency suggest that gp120 binding by DC-SIGN differs from ICAM-3 binding. The three-dimensional structure of DC-SIGN with the pentasaccharide shows that the Val^{351} residue in DC-SIGN participates in carbohydrate binding through van der Waals' interactions [8]. We have demonstrated that this Val^{351} in DC-SIGN denotes the difference between the interaction of DC-SIGN with gp120 and ICAM-3 (Fig. 4) [16]. The Val^{351} residue is important in binding carbohydrates and ICAM-3, but it is not essential for gp120 binding as the $V_{351}G$ mutant of DC-SIGN still interacts with gp120 but is unable to bind ICAM-3 [16]. These data confirm that the interaction of DC-SIGN with ICAM-3, in contrast with that of gp120, is carbohydrate-dependent. Thus, DC-SIGN has a distinct binding site for HIV-1 gp120, which primarily mediates protein-protein interactions [16]. These findings indicate further that in addition to the ligands interacting with the primary binding site centered around the Ca^{2+} at site 2, the ligands form additional contacts with the surface of DC-SIGN, thereby creating different ligand-binding sites for HIV-1 gp120 and ICAM-3. Clustering of DC-SIGN at the cell surface in a novel tetrameric coiled-coil motif is suggested to contribute to a further increase in ligand binding specificity [14]. The different binding sites in human DC-SIGN for ICAM-3 and gp120 are attractive targets for therapeutic intervention of DC-induced immunity and HIV-1 dissemination by DC-SIGN, respectively.

L-SIGN, THE HUMAN DC-SIGN HOMOLOGUE

Initially, analysis of DC-SIGN expression was confused by the presence of the DC-SIGN homologue L-SIGN [53], also called DC-SIGN-related (DC-SIGNR) [54] (**Table 1**). L-SIGN functions as an HIV-1 *trans* receptor similarly to DC-SIGN, but is not expressed by DC (**Fig. 5**) [53]. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of DC with L-SIGN-specific primers, as well as RNA blot analysis with L-SIGN-specific probes, failed to identify any L-SIGN-specific products [53]. Moreover, no staining of L-SIGN on DC was found in vitro or in vivo using L-SIGN-specific antibodies (T.G., unpublished data). Recently, Mummidi et al. [61] showed that mRNA encoding human DC-SIGN2, identical to L-SIGN, was present in DC as detected by RT-PCR. This discrepancy could result from the presence of contaminating L-SIGN-positive cells in DC preparations, as L-SIGN expression can be induced on cells of myeloid origin (T.G., unpublished data).

L-SIGN is expressed specifically by liver sinusoidal endothelial cells (LSEC) [62], a liver-resident APC population [53, 55], and by a specific subpopulation of nonendothelial, macrophage-like cells in lymph nodes (A.E., unpublished results) (Table 1). Liver sinusoids are specialized capillary vessels characterized by the presence of resident macrophages adhering to the endothelial lining. The LSEC-leukocyte interactions, which require expression of adhesion molecules on the cell surfaces, appear to constitute a central mechanism of peripheral immune surveillance in the liver. The MR, MHC class II, and costimulatory receptors such as CD80 and CD86 are known to be expressed on LSEC and to mediate the clearance of many potentially antigenic proteins from the circulation in a manner similar to DC in lymphoid organs [62]. L-SIGN may fit into this category of receptors on LSEC, as its tissue location and ligand-binding properties strongly implicate a physiological role for this receptor in antigen clearance as well as in LSEC-leukocyte adhesion (Fig. 5). The high expression of ICAM-3 on apoptotic cells [63] may provide the means by

TABLE 1. Expression of DC-SIGN Homologues in Human, Primate, and Mouse

Species	Receptor	Tissue expression	Specific expression	Function	References
Human	DC-SIGN	Lymphoid organs, dermis, mucosae, intestine, placenta, lung, blood	DC, Hofbauer cells, decidual Mϕ, alveolar Mϕ	HIV-1, ICAM-2, ICAM-3	[3, 9–13, 41, 44]
	L-SIGN/DC-SIGNR	Liver, lymph node, placenta	LSEC	HIV-1, ICAM-2, ICAM-3	[53–55]
Primate	DC-SIGN"	Lymph node, spleen, mucosae, intestine	DC, Mϕ	HIV-1, ICAM-2, ICAM-3	[56–58]
	L-SIGN	Liver	LSEC	nd	[57]
Mouse	mDC-SIGN	Spleen, lung (mRNA levels)	CD8α-DC > B cells (mRNA levels)	nd	[59, 60]
	SIGNR1	Spleen, liver, lymph node	LSEC, MZM	HIV-1, ICAM-2, ICAM-3	[59] (unpublished results)
	SIGNR2-4	Spleen (R3, R4), testis (R2, R4; mRNA levels)	DC, B and T cells (R2, R3; mRNA levels)	nd	[59]

Mϕ, Macrophages; nd, not determined; MZM, marginal zone macrophages; " Rhesus macaque and chimpanzee.

which these cells are trapped by L-SIGN-expressing cells in the liver and subsequently cleared.

The expression pattern of L-SIGN in liver sinusoids suggests that LSEC, which are in continual contact with circulating leukocytes, can capture HIV-1 from the blood and promote *trans* infection of circulating T cells in the liver. Moreover, previous studies have indicated that LSEC themselves may be susceptible to HIV-1 infection [64]. It is thus possible that L-SIGN promotes infection of these cells, thereby establishing a reservoir for new virus to pass on to T cells, trafficking constitutively through the liver sinusoid. Its expression in lymph nodes suggests that L-SIGN may play an additional role in HIV-1 pathogenesis by promoting HIV-1 infection of T cells in lymph nodes; thus, L-SIGN may be involved in the persistence of chronic HIV-1 infections [53, 55]. L-SIGN could also be involved in vertical transmission because it is expressed in placenta [11, 55]. Additional functional studies are necessary for understanding the physiological role of L-SIGN and its possible role in HIV-1 pathogenesis (Fig. 5).

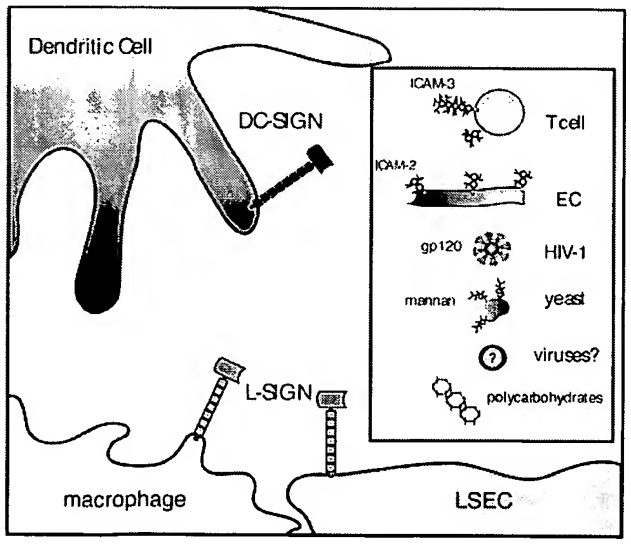

Fig. 5. The C-type lectins DC-SIGN and L-SIGN are expressed by different APC but interact with similar ligands and pathogens. DC-SIGN expression is restricted to DC, whereas L-SIGN is expressed by LSEC and specific macrophages in the lymph nodes but not by DC. Both C-type lectins interact with similar ligands, which indicated that they interact with similar pathogens and host cells. Their affinity for carbohydrates suggests that they may interact with pathogens other than HIV-1.

MURINE HOMOLOGUES OF DC-SIGN

The discovery of DC-SIGN led to more information about processes such as DC migration and initiation of the immune response. However, all experiments to date have been performed in vitro, and a murine in vivo model would be very useful in a more detailed investigation into the in vivo function of DC-SIGN. Recently, five murine DC-SIGN homologues were identified by RT-PCR, and one, murine DC-SIGN, was expressed at high mRNA levels in $CD11c^+$ DC [59, 60] (Table 1). In contrast, the other cDNAs, designated SIGNR1–4, were hardly detectable in DC, but were detected at various mRNA levels in B and T cells by RT-PCR [59] (Table 1).

One of these homologues, SIGNR1, was shown to bind HIV-1 and ICAM-3 [56] (T.G., unpublished results). This murine homologue is expressed specifically by LSEC and by macrophages in spleen and lymph node, but not by DC (T.G., unpublished results) (Table 1), and thus is likely the murine L-SIGN homologue. SIGNR1 has a high affinity for human ICAM-3, yet mice do not express ICAM-3, and therefore binding of SIGNR1 to ICAM-3 is not physiological. However, SIGNR1 is able to interact with murine ICAM-2 (T.G., unpublished results), which is expressed widely on murine lymphocytes [65]. Thus, ICAM-2 could function as the leukocyte ligand for SIGNR1, mediating contact between LSEC and leukocytes in mice. Notably, $CD11c^+$ DC did not interact with soluble ICAM-2 as determined by an in situ adhesion assay (T.G., unpublished results), despite these cells containing high levels of mDC-SIGN mRNA [59, 60]. No specific adhesion

function has been demonstrated for mDC-SIGN, which thus may have a different function to the human homologue. It is also possible that mDC-SIGN does not interact with ICAM-2 but with as yet other unidentified ligands. Thus, the situation in mouse is much more complex than in human, and the expression and function of the murine homologues of DC-SIGN need to be investigated further before a murine model can be investigated productively.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Within the last few years, various C-type lectins have been identified on DC. The pattern of C-type lectin expression depends on DC subset, maturation, and activation state. Although C-type lectin receptors initially were thought to function as scavenger receptors that bind various pathogens upon recognition of particular carbohydrate profiles, it has become clear from work on the C-type lectin DC-SIGN that some C-type lectins may function as adhesion, signaling, or viral receptors.

Many C-type lectins, including DC-SIGN, regulate the capture of different pathogens, based on glycosylation, increasing the diversity and efficiency of antigen recognition by DC. It will be essential to understand how the heterogeneity of glycosylations, recognized by DC-SIGN and other C-type lectins, is regulated at the cellular level and micro-milieu. An intriguing question that arises is: Do oligosaccharides on cellular ligands allow DC migration at specific sites as well as DC interactions to specific T cell subsets? Future research addressing carbohydrate recognition profiles by C-type lectins and the regulation of glycosylation on its cellular ligands will provide insight into how these cell surface receptors mediate cellular interactions and regulate DC function.

Similarly, the great diversity between cytoplasmic tails of DC-expressed C-type lectins that contain internalization motifs, signaling motifs (ITIM and ITAM), hints to distinct regulatory functions within the immune system. As little is known at this moment, it will be challenging to understand the function of C-type lectins in signaling and communication to other cell surface receptors on DC.

Although DC-SIGN is an important adhesion receptor for DC migration and the initial DC-T-cell interaction, recent findings demonstrate that DC-SIGN, similar to other C-type lectins, also functions as an antigen receptor that captures antigens to facilitate processing and presentation by MHC class II molecules. Future research will determine the specific antigens for DC-SIGN. In relation to this, it will be extremely important to understand how HIV-1 is internalized and protected within DC by its binding to DC-SIGN. The mechanisms by which HIV-1 escapes the antigen presentation route of DC and by which it recycles back to the DC membrane and infects T cells in trans are not yet fully understood, and these will be future questions that many researchers will want to answer.

The elucidation of the three-dimensional structure of DC-SIGN cocrystallized with an oligosaccharide identified several important features of ligand binding by DC-SIGN. However, questions remain as DC-SIGN does not have a high affinity to the cocrystallized oligosaccharide. Moreover, binding to its natural ligands may be a result of more complex interactions. This is shown by the results that carbohydrates govern the recognition of ICAM-3 by DC-SIGN, whereas its interaction with gp120 is carbohydrate-independent. Detailed information about the mechanism of ligand binding is crucial for the development of strategies to modulate DC-SIGN function and thus is an important aspect of DC biology.

The DC-SIGN homologue L-SIGN is expressed specifically by LSEC and specific macrophages in the lymph nodes but not by DC. Investigation of its function will provide more insight into the role of these APC to have a tolerizing function in the immune response. To date, the function of DC-SIGN and other DC-expressed C-type lectins is mainly based on in vitro models. To understand the importance of these receptors on DC in driving immune responses, future research should focus on mouse models in which the in vivo function can be addressed. Although the murine homologues of DC-SIGN have been cloned, the complexity of splice variants and cell-specific expression demonstrates that the situation in mouse is more complex than in human. The expression and function of the murine homologues of DC-SIGN need to be investigated further before a murine model can be investigated productively.

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